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Fine structure of γ-irradiated tracheid wall in *Picea abies**

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γ線照射処理した *Picea abies* の仮道管壁の構造*

後藤俊幸・原田　浩・佐伯　浩

CONTENTS

ABSTRACT

When a wood specimen 〔*Picea abies* (L.) KARST〕 was γ-irradiated (655 Mrad), microfibrillar texture as seen in the untreated wood was still clearly observed by replica on the surface of the tracheid, while this wood became non-crystalline with X-ray diffraction. In transverse and longitudinal sections of the tracheid in γ-irradiated wood microfibrils were observed by the method of block negative staining with uranyl acetate. The disintegration of γ-irradated wood produced many short broken microfibrils. The above facts indicate that γ-irradiation produces many defects even within the crystalline region of microfibrils, while retaining their original shape.

要　旨

γ線照射（655 Mrad）処理材〔*Picea abies* (L.) KARST〕の X 線回折図では結晶性ピークを示さないのに，電子顕微鏡でレプリカ法により観察すると，仮道管内壁面には顕著なミクロフィブリルの配向がみられた。

本報では，γ線処理材の仮道管にみられるこのようなミクロフィブリル像の実体を明らかにしようとした。γ線処理材を酢酸ウラニルでブロック負染色法により電子顕微鏡観察すると，仮道管の横断面・縦断面ともにミクロフィブリルが白い斑点状　或いは線状に存在することが確認された。さらにこの試料を解体して観察すると，短破片状のミクロフィブリルがみられた。

以上の結果から，このγ線処理材においては，ミクロフィブリル中に多くの結晶欠陥が存在するが，なおミクロフィブリルの形態は保持されているものと推定される。

* Paper presented at the 24th Annual Meeting of the Japan Wood Research Society at Tokyo, April, 1974.

INTRODUCTION

In previous reports,[1,2] the fine structures of cellulose microfibrils of *Valonia* and wood cell walls, especially their dimensions and the shape of cross-section, were presented. When cross-sections of microfibrils were electron-microscopically observed, those of *Valonia* could be easily observed due to their larger dimensions, while the gelatinous layer of poplar tension wood could not precisely observed simply because it is hard to prepare an ultrathin-section thin enough to observe such small microfibrils as these of 20-40 Å in diameter.

In order to get a thin enough section, strongly γ-irradiated wood sample supplied by Prof. ANTOINE was used as an experimental material. Chemical analysis showed the presence of a trace of cellulose in this γ-irradiated wood,[4] which was also shown to be amorphous by means of X-ray diffractometry. However, when its replica was examined with electron-microscope, the microfibril-like texture could be observed on the lumen surface of tracheid in γ-irradiated wood just as that seen in untreated wood. So far there have been few publications which explain how the microfibrillar texture still remains at this high doses of radiation in spite of chemical deterioration together with low crystallinity.

The purpose of the present work is to investigate the image of the microfibrils in replica, and furthermore to obtain a clue to cellulose microfibril study.

MATERIALS AND METHODS

The materials were γ-irradiated wood [*Picea abies* (L.) KARST] kindly supplied by Prof. ANTOINE, which had been irradiated at 655 Mrad doses by Co 60.[3,4] As a reference a wood specimen of the same species was used which had been stored at the Wood Structure Laboratory of Wood Science and Technology in Faculty of Agriculture of Kyoto University.

The samples were observed both with a scanning electron-microscope (JSM-U3, SEM) and a transmission electron-microscope (JEM-7, TEM), and were also investigated by X-ray diffraction.

A) Electron-microscopic examinations

The samples were coated with carbon and gold before observing with SEM. In TEM the samples were observed with either direct carbon replica,[5] block staining by the method of HEYN,[6] staining and shadowing after removal of resin,[1] or disintegration method.[7]

B) X-ray diffraction method

Gamma-irradiated wood can easily be pulverized simply by pressing with fingers, and 100-200 mesh of powdered sample was used for the untreated wood. These powder samples were then pressed to tablets using a tablet molder for IR analysis which were examined with X-ray diffraction using symmetrical-reflection technique.[7] The samples were also investigated in their block shape by the same technique which were scanned in both parallel and perpendicular directions to cell axis.

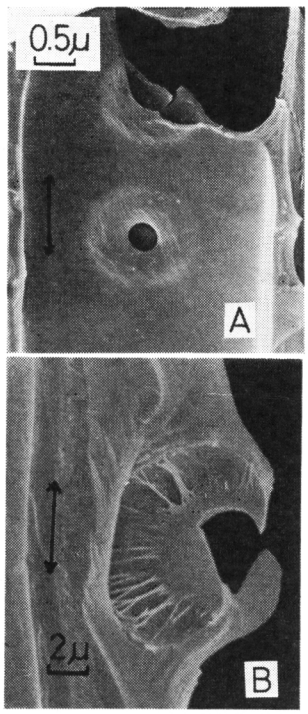


Fig. 2. Scanning electron micrographs of γ-irradiated wood. The arrows show the direction of tracheid axis.
(A) Inner surface of the tracheid wall showing the microfibrillar texture.
(B) Bordered pit showing the radiated microfibrils in margo.

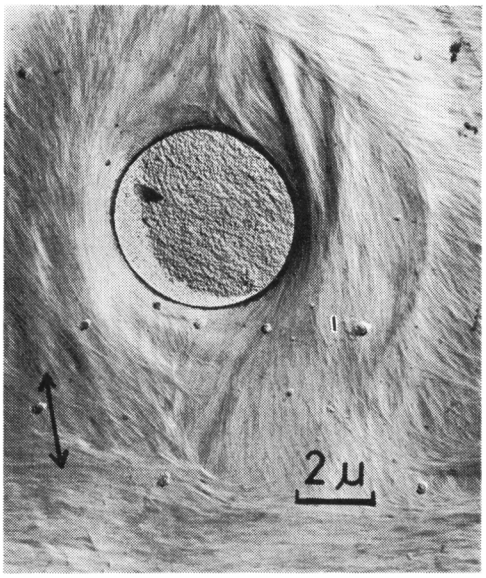


Fig. 3. Direct carbon replica of γ-irradiated wood showing clear microfibrillar texture on the surface of the tracheid wall. The arrow shows the direction of tracheid axis.

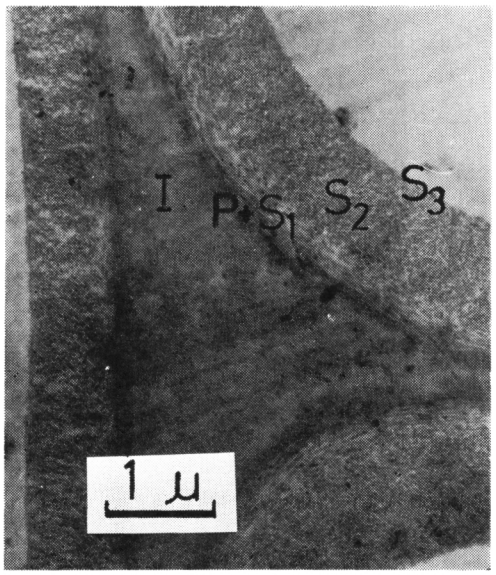


Fig. 4. Electron micrograph of transverse section of γ-irradiated tracheids. Negatively stained with uranyl acetate. Black area is stained. White dots and lines in the wall indicate microfibrils.

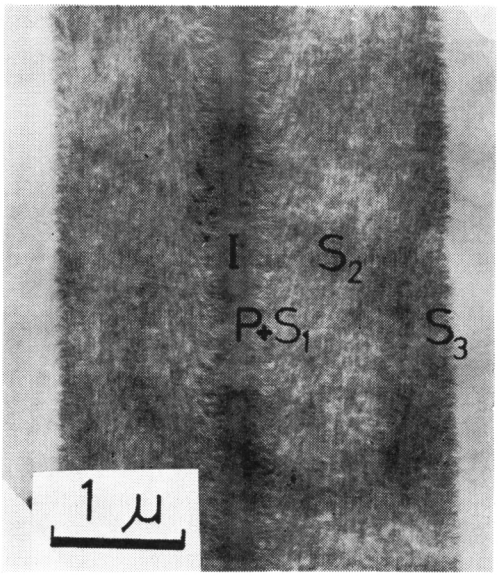
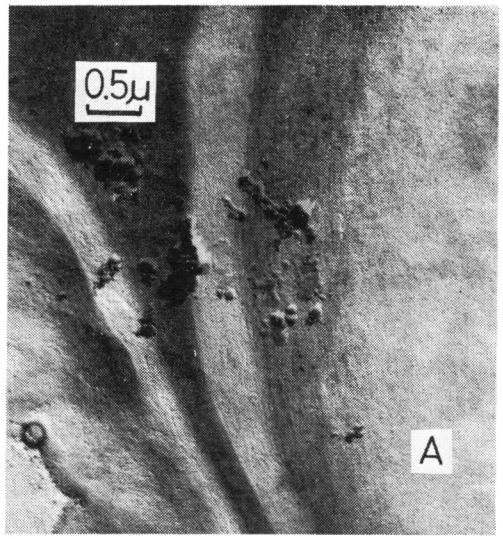


Fig. 5. Electron micrograph of longitudinal section of γ-irradiated tracheids. Negatively stained with uranyl acetate.



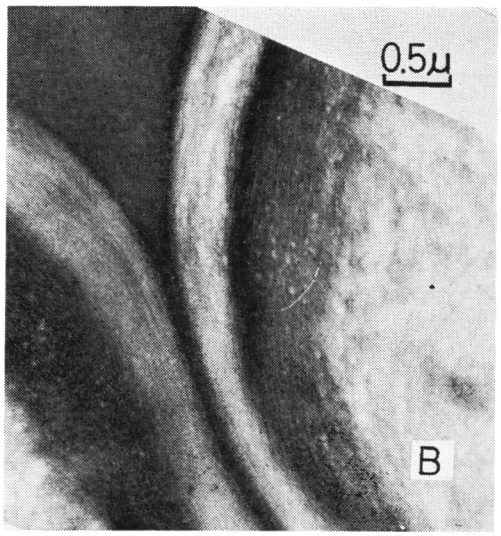


Fig. 6. Electron micrographs of nearly transverse sections of γ-irradiated wood. After methacrylate resin is removed, Pt-C shadowed (A) and negatively stained with uranyl acetate (B). The photos show the closely packed cell wall.

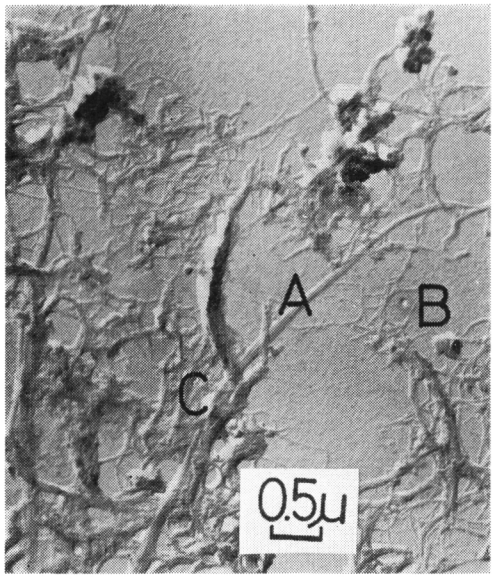


Fig. 7. Microfibrils of untreated wood. Shadowed with Pt-C. A and B show the bundle of long microfibrils and isolated short microfibrils, respectively, C the structure-less substances such as hemicelluloses and lignin.

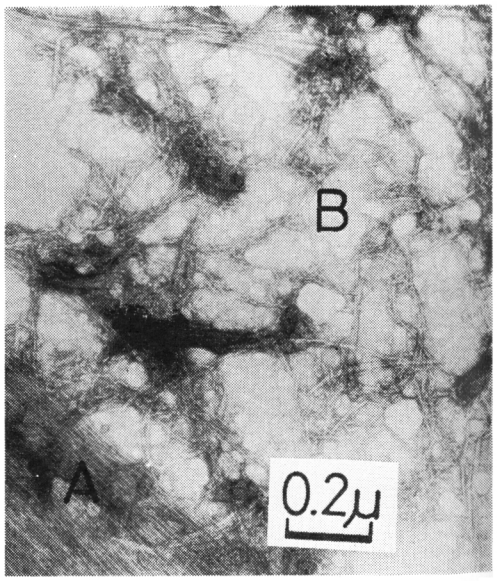


Fig. 8. Microfibrils of untreated wood. Negatively stained with uranyl acetate. The formvar film covered by evaporated carbon was rendered hydrophilic by exposure to glow discharge. Negatively stained with uranyl acetate. See the legend of Fig. 7.

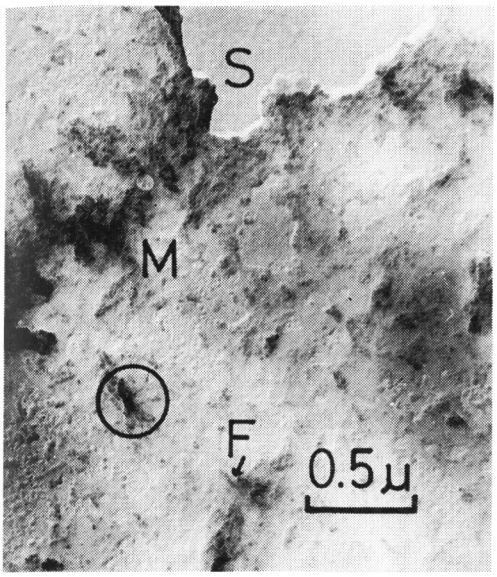


Fig. 9. Disintegrated fragments of γ-irradiated wood. Shadowed with Pt-C. The circle area shows the bundles of microfibrils. S : supporting film, M : matted substances, F : shortly cut microfibrils.

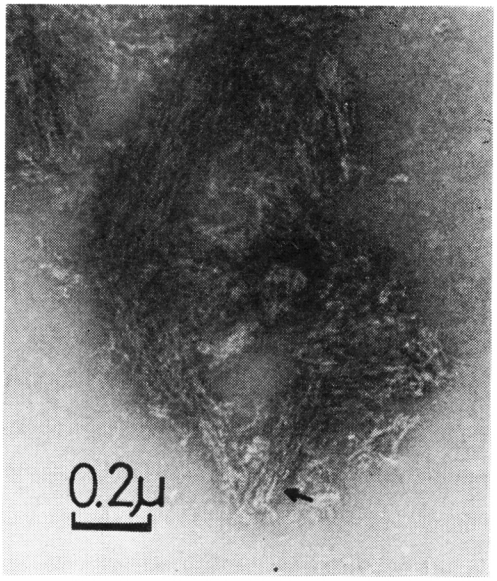


Fig. 10. Microfibrils of γ-irradiated wood. The arrow shows a microfibril with many defects along the longitudinal direction.

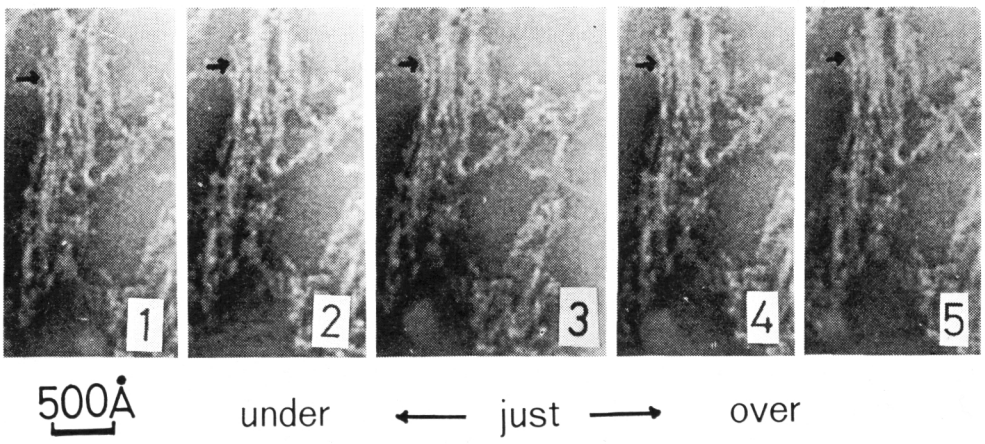


Fig. 11. A through-focus series of electron micrographs of the microfibrils of γ-irradiated wood. Stained with uranyl acetate. Photo 3 shows near-focused, 1 and 2 under-focused, and 4 and 5 over-focused. In all photos, even in photo 3, microfibrils remain their original granularity.

RESULTS

1) Examinations with X-ray diffraction method

In chemical analysis of this γ-irradiated wood[3], while ethanol-benzene as well as water extractives increased, the cellulose content remarkably decreased. On the other hand lignin seemed to be stable to γ-ray. The degree of polymerization of cellulose decreased from 1400 to 13 after γ-irradiation. The viscometric study indicates that γ-irradiated wood does not have high DP cellulose which have long chain molecules.

Figure 1 shows X-ray diffractograms of powder samples. Untreated wood shows the crystalline pattern of cellulose I : a doublet of the (101) and ($10\bar{1}$) reflections at 16-18° (2θ), a strong peak of the (002) reflection near 22.6° and a weak peak of the (040) reflection at 34.5° can be detected. However, in γ-irradiated wood, crystalline peaks as observed in untreated wood do not appear, but an amorphous halo exists with a maximum around 20-21°. This is confirmed through X-ray scanning of the block samples in both parallel and perpendicular directions to the longitudinal axis of the fiber.

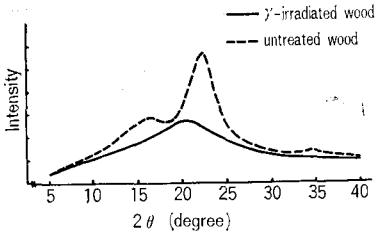


Fig. 1. X-ray diffractograms of γ-irradiated wood and untreated wood (powder technique) ; corrected for air scattering.

According to viscometric and X-ray diffraction studies, γ-irradiated wood seems to have short cellulose molecules which are non-crystalline.

2) Observations of the inner surface of the wood cell wall irradiated with γ-ray

In the scanning electron-micrographs (Figure 2), the microfibrillar texture is clearly observed on the surface of the tracheid wall (Fig. 2-A), and in the region of margo of bordered pit membrane (Fig. 2-B) the bundles of radiated microfibrils can be seen as well as in untreated wood, although the cleaved surface of cell wall may be seen showing a fracture of fragile material.

In the observation of replica with TEM (Figure 3), γ-irradiated wood has also clear microfibrillar texture, showing that there is no difference at least in microfibrillar texture between γ-irradiated and untreated woods.

3) Observations of the section in γ-irradiated wood

Small blocks of γ-irradiated wood were immersed in aqueous solution of uranyl acetate and embedded in Epon 812 according to HEYN's procedure[6]. In the transverse section of the cell wall (Figure 4) white dots and lines are observed, of which the former are seen in $P+S_1$ and S_3 layers, while the latter are in S_2 layer. Figure 5 is a longitudinal section of the same wood. In this figure, $P+S_1$ layers are seen as white dots. Considering the transverse and longitudinal sections together, these white dots and lines may be oriented almost perpendicular to the cell axis. In S_2 layer white lines are seen in Fig. 5 showing that may be oriented nearly parallel to the cell axis. In S_3 layer 2-3 lamellae can be seen and white dots and lines are oriented perpendicular to cell axis.

The white dots and lines described above are supposed to be, from the principle of

negative staining[1,6], unstained microfibrils showing that the microfibrils detected by electron microscopy exist even in γ-irradiated wood as well as in untreated wood.

However, to eliminate the possibility that these white dots and lines are either void or empty space, the following procedure was examined. When γ-irradiated wood was immersed in water for a long time, the shape of the cell walls changed because of its larger content of water extractives[9]. Therefore the air-dried samples were embedded directly in methacrylate without dehydration. Ultrathin-sections were cut and shadowed with Pt-C (Figure 6A) or negatively stained with uranyl acetate (Figure 6B) after removing the resin with toluene. These two micrographs show the same area in the serial sections. In the cell wall of (A) the microfibrillar texture can be seen particularly in S_1 and outer parts of S_2, and the cell wall may be packed closely and the oriented microfibrils which are shown as white lines are seen clearly in (B). In Fig. 6 the microfibrils in S_2 layer may be more or less inclined to the cell axis. According to the above results, the white dots and lines in the ultrathin-sections of block stained specimen in Figs. 4 and 5 are coincident with the microfibrils in these micrographs of Fig. 6, but the diameters of the microfibrils in the former figures may be a little greater. This confirms agein that γ-irradiated wood has the same microfibrilar texture as that of untreated wood.

4) Microfibrillar structure

Untreated wood was disintegrated for a prolonged period with a homogenizer. A drop of the suspension which was mounted on a grid coated with formvar film and shadowed with Pt-C is seen in Figure 7. Cellulose microfibrils appear as long lines (A) or shortly cut fragments (B). The structure-less substances (C) are supposed to be matrix substances such as hemicelluloses and lignin. When the same suspension is observed after staining with uranyl acetate (Figure 8), long cellulose microfibrils are seen together with short broken ones which may be caused by the long and vigorous treatment.

Gamma-irradiated wood was also electron-microscopically examined. This wood could be easily broken in a few minutes by treatment with ultrasonication (Bransonic 12 ; Frequecy : 50 KHz, Power : 50 W) which was ineffective with untreated wood. The suspension of γ-irradiated wood was mounted on a grid in the same way as untreated wood. Figure 9 shows shadowed specimen which is in matted form except in the parts of supporting film (S). In the matted substances (M) short microfibrils (F) seem to exist. These matted parts are supposed to be lignin, hemicelluloses or fragments which may originate from cellulose microfibrils but do not retain the original shape of microfibrils.

This disintegrated specimen of γ-irradiated wood with negative stain is seen in Figure 10, showing that microfibrils are uniformly broken to short fragments. The cluster in Fig. 9 (encircled area in the figure) may be a bundle of shortly cut microfibrils.

In order to observe these microfibrils at high magnification, the "through focus" method was applied where electron micrographs were taken stepwisely from under-focus through just-focus to over-focus. This method is known as being very useful when one uses electron microscope at high magnification to eliminate false granularity[8,9].

As an example, Figure 11 shows a series of through-focus electron-micrographs of γ-irradiated wood. Photo 3 is the near-focus micrograph. Even at near-focus, the micro-

fibrils of γ-irradiated wood are cut short and their size is about 20 Å in width and 20-40 Å in length showing that microfibrils were cut to relatively the same length of fragments by γ-radiation.

DISCUSSION

The observations of γ-irradiated wood with electron microscopy indicate that even in wood irradiated at a dose of 655 Mrad microfibrillar textures exist as in untreated wood, and these observations are in agreement with those of Burmester[10] and Chiaveriana et al.,[11] who claimed that the irradiation (at less than about 100 Mrad doses) never induced fundamental changes of microfibrillar structure.

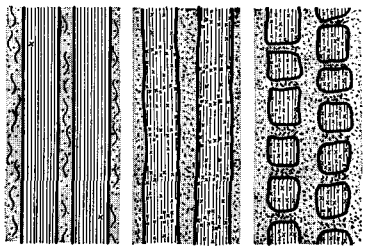


Fig. 12. Diagramatic representation of γ-irradiated microfibrils. (A) untreated, (B) γ-irradiated, (C) γ-irradiated and mild ultrasonicated. F : microfibril, M : matrix substances, X : crystalline defect, ▒ : stain.

The reasons seem to be as follows. As is shown in Figure 12 (A) the core of the microfibrils of untreated wood is almost perfectly crystalline though it has a few dislocations (indicated X in the figure) which may be caused by chain-ends, and the stain indicated by the dotted screen cannot penetrate into the core. However, when cellulose is irradiated with high doses of γ-radiation, it is attacked uniformly both in crystalline and in amorphous regions,[12] and many crystalline defects might be produced even at the core of the microfibrils. These defects are thought to be not only the break-down of cellulose chains but also some modifications of cellulose caused by dehydrogenation.[13] The X-ray crystallographic examinations of the γ-irradiated wood are also in keeping with the above interpretation where the X-ray pattern seemed amorphous, and yet the microfibrils retain their original shape (Fig. 12-B). Even a small external force such as a few minutes of ultrasonication can reveal that microfibrils are only arrays of short fragments as a whole which are attacked initially in the area of relatively many defects (Fig. 12-C). The fragments of the microfibrils are relatively uniform, which may indicate the inner structure of the original microfibrils or only an artefact caused by the random attack of irradiation. This point needs further study.

The above discussion may necessitate the modification of the concept of negative staining. When microfibrils are observed, negative stains such as uranyl acetate and phosphotungstate acid have been used widely, especially of the former,[14] because of their small ionic diameters. The microfibril width measured by electron micrographs is in the range of micelle width estimated by peak broadening of X-ray diffraction.[7] So, the negative stain has been considered to impregnate the whole region except the crystalline region, and this negative staining technique has been used in cellulose materials to differentiate the crystalline region as translucent part from the other stained (opaque) region in electron-micrographs. However, in γ-irradiated wood, though amorphous in X-ray diffraction, the

microfibrils are seen with negative staining by electron-microscopy, which may indicate that the cellulose microfibrils seen with negative staining do not always correspond to the micelles in X-ray crystallography. So it may well be concluded that negative stains can neither penetrate the crystalline region nor the closely packed regions even though some penetrated space may exist within these regions. This again needs further study.

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